REMARKS

Claims 1-12 have been cancelled without prejudice or disclaimer. Claims 13-17 remain pending. Reconsideration and allowance of claims 13-17 in view of the following facts and arguments respectfully are requested.

Objection/Rejection Withdrawn

Applicants thank the Examiner for withdrawing rejections to claims 17 and 11-17, made under 35 U.S.C. § 112, second paragraph, in view of the amendment and arguments submitted.

Rejections Maintained

On page 2-6 (item # 3) of the Advisory Action, the Examiner has maintained the rejection of claims 13-17 under 35 U.S.C. § 112, first paragraph, and alleged lack of written description of cysteine desulfinase genes. Applicants respectfully disagree with the Examiner and submit that the Examiner has not established a *prima facie* case of non-enablement. Accordingly, Applicants submit that their application is in full compliance with 35 USC § 112.

It is respectfully noted that section 112 mandates that patent applications contain the "manner and process of making and using" the invention. The courts have considered applications in compliance with section 112 where the person of skill in the art can practice the invention without undue experimentation. *See Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 94 (Fed. Cir. 1986). The test is not whether experimentation is necessary, but whether any experimentation would be undue in view of what type and amount of experimentation is usual in that particular field. *See* MPEP §§ 2164.05 (a-b), 2164.06 (Rev. 1, February 2003). Routine design choices cannot be equated with non-enablement.

Thus, the burden to establish an enablement rejection rests with the Examiner. *See* MPEP §§ 2164.01; 2164.04 (Rev. 1, February 2003). As explained by the Federal Circuit in considering the intertwined issues of enablement and utility:

> "[I]t follows that the PTO has the initial burden of challenging a presumptively correct assertion of utility in the disclosure. Only after the PTO provides evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility does the burden shift to the applicant to provide rebuttal evidence sufficient to convince such a person of the inventor's asserted utility. [ ] Taking these facts — the nature of the invention and the PTO's proffered evidence — into consideration we conclude that one skilled in the art would be without basis to reasonably doubt applicants' asserted utility on its face. The PTO has not satisfied its initial burden. Accordingly, applicants should not be required to substantiate their presumptively correct disclosure to avoid a rejection under the first paragraph of § 112."

See *In re Brana*, 34 USPQ2d 1436, 1441 (Fed. Cir. 1995) (emphasis added), citing *In re Marzocchi*, 169 USPQ 367, 369-70 (CCPA 1971).

Applicants submit that the Examiner has not met this burden, as explained below.

In making the rejection, on page 2 of the Advisory Action, the Examiner has misunderstood the claimed invention and stated that "claims broadly encompass a genus of cysteine desulfinase genes" and "the specification does not place any structure limitations on the cysteine desulfinase gene." Applicants point out that the claims, for example, the independent claim 1 pertains to "a method of producing pertussis toxin, comprising cultivating *Bordetella pertussis* bacteria that lack cysteine desulfinase activity." Unlike the Examiner's interpretation, applicants clarify that according to the claims, cultivation of *Bordetella pertussis* bacteria that lack cysteine desulfinase activity is necessary. Therefore, the claimed methods do not require the cysteine desulfinase gene or any structure/variant thereof. Applicants further clarify

that the methods require "*Bordetella pertussis* bacteria that lack cysteine desulfinase activity" and the specification provides adequate written description of various methods (see for example, paragraph 40-44 of the specification) of obtaining such "bacteria." Also see, for example, page 13, paragraph 40 of the specification, describing "such knockout mutants may be prepared by anyone of a number of different methods." The application specifically provides several examples, including cultivating the bacteria in presence of cysteine desulfinase antisense nucleotides (see for example, claim 16) and more specifically a mutant strain (see for example, claim 15).

On page 3 of the Advisory Action, again, the Examiner also has misunderstood the claimed invention and stated that "the claimed genus includes cysteine desulfinase genes produced by other microorganisms" and cited Mihara *et al.*, as an example. Applicants refer to above paragraph and reiterate that the methods according to the invention include "*Bordetella pertussis* bacteria that lack cysteine desulfinase activity" and do not include "cysteine desulfinase genes produced by other microorganisms." There would be no reason to recite in the claims a *Bordetella pertussis* bacteria that lacks the *E. coli* cysteine desulfinase gene, for example.

Applicants indicate that Mihara *et al.* is incorporated in the application (see paragraph 65 of the specification) and that the specification discloses how the knowledge of *NIFS*s sequences from other microorganisms listed in "Table III of Mihara *et al.*" could be used by skilled artisan to practice the invention.

Applicants also point out that none of the microorganisms listed in "Table III of Mihara *et al.*" produce pertussis toxin. There is no known bacteria other than *Bordetella pertussis* that can produce the pertussis toxin and demonstrate cysteine desulfinase activity. The claimed methods also do not include other microorganisms or "genes of other microorganisms and genes yet to be discovered." However, sequence information related to cysteine desulfinase gene for homologous recombination (see specification paragraphs 40 and 65) or antisense hybridization (see specification paragraphs 44 and 45) are readily available in GenBank

database. Applicants point out that the gene to be knocked out may be any gene provided that at least some sequence information on the DNA to be disrupted is available to use in the preparation of the knockout construct. Applicants indicate that for homologous recombination the "homology must be at least two base pair long" (see paragraph 40 of the specification), the hybridization conditions are not very stringent that it can work even if the sequences are not identical.

Applicants also indicate that the DNA sequence to be used to knockout a selected gene can be obtained using methods well known in the art, such as those described by Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1989) (see paragraph 42 of the specification). Such methods include, for example, screening a genomic library with a cDNA probe encoding at least a portion of the same gene in order to obtain at least a portion of the genomic sequence. Alternatively, if a cDNA sequence is to be used in a knockout construct, the cDNA may be obtained by screening a cDNA library with oligonucleotide probes or antibodies (where the library is cloned into an expression vector). If a promoter sequence is to be used in the knockout construct, synthetic DNA probes can be designed for screening a genomic library containing the promoter sequence (see paragraph 40 of the specification for reference to US Patent No. 5,557,032).

Thus, the skilled artisan will be able to practice the invention by using the cysteine desulfinase gene sequence information that are publicly available in the GenBank database and the disclosure of the invention.

Furthermore, applicants refer to other methods, for example, transposon mutagenesis or irradiation, to knockout a known or unknown gene to obtain a defective phenotypes with or without the knowledge of structure/sequence details. For example, Transposon mediated *comB* gene knockout mutation resulted in transformation-defective phenotypes. Involvement of the *comb* gene in genetic transformation was not known at the time. Thus, the sequence/structure of the gene involved was not known and was not necessary to obtain the transformation-defective mutant (See attached abstract, Hofreuter *et al. Mol Microbiol.* 1998 June;

28(5):1027-38). Irradiation induced gene-knockout resulted in Ataxia-telangiectasia mutated gene (ATM) functions knockout phenotype, such as inhibition of cyclin dependent kinase 2 (CDK2) was abolished. Involvement of CDK2 in ATM function was not known at the time. Thus, the sequence/structure of the gene involved was not known and was not necessary to obtain the ATM function knockout mutant (See attached abstract, Zhou *et al. J Tongji Med Univ.* 2000; 20(4):273-6).

Therefore, it is possible to make a cysteine desulfinase knockout mutant without knowing the gene sequence. For example, using the transposon mutagenesis method, a transposon (for example, Tn5) can be integrated at random into the genome. In the case of Tn5, one skilled in the art would select for a kanamycin resistant *Bordetella*, a marker encoded by the transposon, and screen the transposon mutants for those lacking the cysteine desulfinase activity. See Mosqueda *et al.* (*J Bacteriol.* 2000 February; 182(4):937-43, Abstract attached) for an example of Tn5-induced mutagenesis of a target gene without knowing the sequence and structural details. The Tn5 induced knockout mutation identified a second toluene efflux system for toluene metabolism. A *ttgD* gene knockout mutant was characterized for toluene tolerance. The *ttgD* gene was not known at the time for toluene resistance thus, the sequence/structure of the gene was not necessary to obtain the knockout mutant.

Therefore, structural details are not necessary in order to obtain a knockout mutant. However, applicants need not rely on this approach because the specification does disclose the sequences of the *Bordetella pertussis* cysteine desulfinase gene.

Moreover, applicants demonstrate below that the requirements set forth by the Examiner find correspondence in the specification.

In making the rejection, on pages 4-6 of the Advisory Action, the Examiner stated that the specification "broadly describes a genus of cysteine desulfinase genes." While there is a broad and detailed description in the specification, claim 14 recites a *Bordetella pertussis* cysteine desulfinase gene. Figures 7A-7C disclose polynucleotide and amino acid sequence for a *Bordetella pertussis* cysteine

desulfinase gene. Paragraph 58 of the specification teaches how to clone a *Bordetella pertussis* cysteine desulfinase gene, including a disclosure of appropriate primers. Paragraph 65 of the specification discloses *NIFS*s genes in other microorganisms, including methods to obtain sequence information by PCR and cloning. A transformed *Bordetella pertussis* knockout mutant, which lacks cysteine desulfinase activity, has been deposited at the American Type Culture Collection. See paragraph 60 of the specification.

Applicants submit that the above discussion shows that it is clear that applicants had possession of the subject matter claimed. Given the correspondence and applicants' identification of this correspondence, a heavy burden is placed upon the Examiner to reject the claims given that the specification is presumed adequate. *See* MPEP § 2163.04 (Rev. 1, February 2003). Applicants therefore request withdrawal of the rejection.

REQUEST

Applicants submit that the claims are in condition for allowance, and respectfully request favorable consideration to that effect. The Examiner is invited to contact the undersigned at (202) 912-2000 should there be any questions.

Respectfully submitted,

August 10, 2004
Date

John P. Isacson
Reg. No. 33,715

Heller Ehrman White & McAuliffe LLP
1666 K Street, N.W., Suite 300
Washington, D.C. 20006
Telephone: (202) 912-2000
Facsimile: (202) 912-2020
Customer No. 26633